

## APPENDIX A

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ZAGREB  
YUGOSLAVIA  
1961

Croat. Chem. Acta 33 (1961) No. 3

(Pag. 99—156)

15.01.1962.

CCA-223

545.844:547.915

### Chromatography of the Lipide Bases on Paper Impregnated with Silicic Acid\*

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Received October 13, 1961

Chromatography of various long-chain aliphatic  $\alpha$ -amino alcohols,  $\alpha$ -amino ketones and  $\alpha$ -diamines containing 18 and 20 carbon atoms on paper impregnated with silicic acid is described. Most of these compounds — which are listed in Table I — are of biological importance.

In connection with our investigation of the sphingolipids and other complex lipides, the need arose for a paper chromatographic procedure for separating certain long-chain aliphatic amino compounds. We have obtained the most satisfactory results by the use of paper impregnated with silicic acid.<sup>1,2</sup> This technique has been applied to various  $\alpha$ -amino alcohols,  $\alpha$ -amino ketones and  $\alpha$ -diamines of natural and synthetic origin possessing a chain-lengths of 18 and 20 carbon atoms. There are presently no published reports on the chromatographic separation and characterization of these compounds, with the exception of sphingosine (2-amino-1,3-dihydroxy-4-octadecene)<sup>3,4,5</sup> and dihydrosphingosine (2-amino-1,3-dihydroxyoctadecane).<sup>4,6</sup> The investigation described here is limited to the compounds containing 18 and 20 carbon atoms with regard to their biological significance.

As shown in Table I, the amino compounds investigated could be differentiated as to the number of the amino groups and hydroxy groups. Thus, the increasing number of hydroxy groups caused a decrease of the relative movement of the base. Owing to this fact, for instance, a mixture of  $C_{18}$ -sphingine (1 OH),  $C_{18}$ -sphingosine or  $C_{18}$ -dihydrosphingosine (2 OH) and  $C_{20}$ -phyto-sphingosine (3 OH) could easily be separated. Another example is a mixture of  $C_{20}$ -phytosphingosine and its anhydro derivative which results by methanolysis of yeast cerebrin. It is difficult to separate those two compounds on a preparative scale. However, the clean-cut separation on paper offers a reliable way to detect the purity of a product. Similarly, a mixture of the synthetic long-chain diamines<sup>7</sup> containing a small proportion of the corresponding amino alcohols can easily be resolved by paper chromatography. On the other hand, there is little difference between the  $R_f$  values of the monohydroxy compounds of the  $C_{18}$  and  $C_{20}$  series, whose mobilities with the referred solvent system proved to be closely similar. Under the experimental conditions given the average deviation in  $R_f$  values did not exceed  $\pm 0.03$ .

\* Presented at the I. Congress for Pure and Applied Chemistry of Yugoslavia, Zagreb, June 1960. Abstracts of Papers p. 135.

Table I  
R<sub>f</sub> Values of the Long-Chain Amino Compounds

Compound	$R_f$	Number of functional groups	
		NH <sub>2</sub>	OH
C <sub>18</sub>			
2-Amino-1-hydroxyoctadecane (C <sub>18</sub> -Sphingine)	0.34	1	1
2-Amino-3-hydroxyoctadecane	0.32	1	1
2-Amino-1,3-dihydroxyoctadecane (C <sub>18</sub> -Dihydrosphingosine)	0.29	1	2
2-Amino-1,3-dihydroxy-4-octadecene (C <sub>18</sub> -Sphingosine)	0.29	1	2
2-Amino-3-octadecanone . HBr	0.38	1	—
2,3-Diaminoöctadecane	0.25	2	—
C <sub>20</sub>			
2-Amino-1-hydroxyeicosane (C <sub>20</sub> -Sphingine)	0.35	1	1
4-Amino-5-hydroxyeicosane	0.42	1	1
3-Amino-4-hydroxy-2-methyl- nonadecane	0.42	1	1
2-Amino-1,3,4-trihydroxyeicosane (C <sub>20</sub> -Phytosphingosine)	0.23	1	3
4-Amino-5-eicosanone.HBr	0.49	1	—
3-Amino-2-methyl-4-nonadecanone. HBr	0.49	1	—
4,5-Diaminoeicosane (Necrosamine)	0.29	2	—
3,4-Diamino-2-methylnonadecane	0.29	2	—
2-Amino-1,4-anhydro-1,3,4- trihydroxyeicosane (C <sub>20</sub> -Phytosphingosine Anhydro Base)	0.35	1	1

## EXPERIMENTAL

## Materials

2-Amino-1-hydroxyoctadecane (C<sub>18</sub>-sphingine)<sup>9</sup>, 2-amino-1-hydroxyeicosane (C<sub>20</sub>-sphingine)<sup>9</sup>, 2-amino-3-hydroxyoctadecane<sup>10</sup>, 4-amino-5-hydroxyeicosane<sup>11</sup>, 3-amino-4-hydroxy-2-methylnonadecane<sup>11</sup>, 2-amino-3-octadecanone hydrobromide<sup>10</sup>, 4-amino-5-eicosanone hydrobromide<sup>7</sup>, 3-amino-2-methyl-4-nonadecanone hydrobromide<sup>7</sup>, 2,3-diaminooctadecane<sup>10</sup>, 4,5-diaminoeicosane (necrosamine)<sup>7</sup> and 3,4-diamino-2-methylnonadecane<sup>7</sup> were synthetic products. 2-Amino-1,3-dihydroxy-4-octadecene (C<sub>18</sub>-sphingosine)<sup>12</sup> and 2-amino-1,3-dihydroxyoctadecane (C<sub>18</sub>-dihydrosphingosine)<sup>12</sup> were prepared from bovine brain lipides. 2-Amino-1,3,4-trihydroxyeicosane (C<sub>20</sub>-phytosphingosine)<sup>13</sup> and 2-amino-1,4-anhydro-1,3,4-trihydroxyeicosane (C<sub>20</sub>-phytosphingosine anhydro base)<sup>13</sup> were obtained from yeast cerebrin.

## Methods and Procedure

Whatman No. 1 filter paper was impregnated with silicic acid (Mallinckrodt 100 mesh for chromatography or silicic acid prepared in this laboratory from sodium silicate) essentially as described by Marinetti *et al.*<sup>1,2</sup> Chromatograms were carried out at 25° by the ascending technique in glass cylinders. The developing solvent system was di-iso-butyl ketone — acetic acid — water 40:25:5 (vol/vol). This system showed to be very useful for the separation of phosphatides.<sup>1,2</sup> The time of the

chromatographic run was 15—ninhydrin. The R<sub>f</sub> values thus

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## Kromatografija lipoidnih

Tehnika kromatografije jenjena je na niz alifatskih i sintetskog porijekla s lancir prikazane su u Tablici I.

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pounds

	Number of functional groups	
	NH <sub>2</sub>	OH
	1	1
	1	1
	1	2
	1	2
	1	—
	2	—
	1	1
	1	1
	1	1
	1	3
	1	—
	1	—
	2	—
	2	—
	1	1

amino-1-hydroxyeicosane  
 -5-hydroxyeicosane<sup>11</sup>, 3-  
 anone hydrobromide<sup>10</sup>, 4-  
 nadeconone hydrobromi-  
 ine<sup>7</sup> and 3,4-diamino-2-  
 -dihydroxy-4-octadecene  
 3,18-dihydrosphingosine<sup>12</sup>  
 roxyeicosane (C<sub>20</sub>-phyto-  
 sane (C<sub>20</sub>-phytosphingo-

ic acid (Mallinckrodt 100  
 laboratory from sodium  
 natograms were carried  
 The developing solvent  
 : 5 (vol/vol). This system  
 des.<sup>1,2</sup> The time of the

chromatographic run was 15–20 hours. The lipid bases on paper were detected with ninhydrin. The R<sub>f</sub> values thus obtained are listed in Table I.

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## IZVOD

Kromatografija lipoidnih baza na papiru impregniranom silicijevom kiselinom

B. Palameta i M. Proštenik

Tehnika kromatografije na papiru impregniranom silicijevom kiselinom primijenjena je na niz alifatskih  $\alpha$ -aminoalkohola,  $\alpha$ -aminoketona i  $\alpha$ -diamina prirodnog i sintetskog porijekla s lancima od 18 i 20 ugljikovih atoma. Dobivene R<sub>f</sub> vrijednosti prikazane su u Tablici I.

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Primljeno 13. listopada 1961.

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